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Screening for mutations in the uroporphyrinogen decarboxylase gene using denaturing gradient gel electrophoresis. Identification and characterization of six novel mutations associated with familial PCT.

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The two porphyrias, familial porphyria cutanea tarda (fPCT) and hepatoerythropoietic porphyria (HEP), are associated with mutations in the gene encoding the enzyme uroporphyrinogen decarboxylase (UROD). Several mutations, most of which are private, have been identified in HEP and fPCT patients, confirming the heterogeneity of the underlying genetic defects of these diseases. We have established a denaturing gradient gel electrophoresis (DGGE) assay for mutation detection in the UROD gene, enabling the simultaneous screening for known and unknown mutations. The established assay has proved able to detect the underlying UROD mutation in 10 previously characterized DNA samples as well as a new mutation in each of six previously unexamined PCT patients. The six novel UROD mutations comprise three missense mutations (M01T, F229L, and M324T), two splice mutations (IVS3-2A-->T and IVS5-2A-->G) leading to exon skipping, and a 2-bp deletion (415-416delTA) resulting in a frameshift and the introduction of a premature stop codon. Heterologous expression and enzymatic studies of the mutant proteins demonstrate that the three mutations leading to shortening or truncation of the UROD protein have no residual catalytic activity, whereas the two missense mutants retained some residual activity. Furthermore, the missense mutants exhibited a considerable increase in thermolability. The six new mutations bring to a total of 29 the number of disease-related mutations in the UROD gene. The DGGE assay presented greatly improves the genetic diagnosis of fPCT and HEP, thereby facilitating the detection of familial UROD deficient patients as well as the discrimination between familial and sporadic PCT cases. Copyright 1999 Wiley-Liss, Inc.